Brief Notes

Iron Particles in Normal Erythroblasts and Normal and Pathological Erythrocytes. By Marcel C. Bessis and Janine Breton-Gorius. (*From Centre National de Transfusion Sanguine, Paris, France.*)*

Bone marrow for this investigation was obtained from human ribs in the course of surgical intervention. Small fragments were immediately placed in Palade's or Dalton's fixative and the usual procedure for fixation, dehydration, and embedding in butyl methacrylate was followed. The Porter-Blum microtome was used for sectioning and the R.C.A. EMU 3B for observation.

We have previously shown (1) that approximately 30 per cent of the sections of polychromatophilic normoblasts contain circumscribed areas of high electron density within the cytoplasm. These areas vary in size from 0.1 to 0.4 μ (Fig. 2) and are composed of from 50 to 400 iron-containing granules in the order of 40 to 100 A which are spaced approximately 40 A apart. Occasionally these areas are limited by a dense cytoplasmic zone which in some instances has the appearance of a membrane. The presence of this membrane-like structure (*cf.* Fig. 3 *e*) is more common on the edge of the cell. Isolated granules and small groups of 3 or 4 granules may also be noted. The hypothesis that these granules contain iron (in a combined form) is supported by the following observations: 1. These areas are identifiable as the areas in siderocytes which have been characterized as iron-containing by cytochemical tests; 2. Their electron density is compatible with the presence of a metal (formol fixation instead of osmium tetroxide eliminates the possibility of their representing a lipide-containing substance); 3. Their quantity parallels the amount of non-hemoglobin iron; and 4. Microscopically they resemble the ferritin molecules which Farrant (3) has obtained from horse spleen.

The disappearance of these areas parallels hemoglobin synthesis. As synthesis nears completion and the cell approaches its full complement of hemoglobin only a few isolated granules may be seen. The normal reticulocyte may contain an occasional dense area (Fig. 2). On the other hand, in hemolytic anemia of the newborn the number of areas is considerably increased in the reticulocyte as well as in the erythrocyte. The total number of cells containing the dense areas is also increased. In thalassemia approximately 80 per cent of the erythrocytes have iron-containing granules (Fig. 4). The granules are often included in a vacuole; however they may be dispersed due to rupture of the vacuole.

Palade (5) noted in the macrophage of rat spleen a round body with a granular structure of high electron density. He believes this to be a product of the digestion of phagocytosed elements. In human bone marrow we have observed numerous dense granules thought to be degradation products of phagocytosed erythrocytes. These granules are often liberated by fragmentation of the cytoplasm of the

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macrophage (Fig. 1). It is of interest to note that the macrophages of the marrow are generally near islands of erythroblasts. Our knowledge of metabolic iron conservation renders it reasonable to assume that these granules enter the erythroblasts, thus completing the cellular iron cycle.

In a preliminary publication (2) we have described the mechanism by which we think the iron-containing granules pass from the macrophage to the erythroblast. At a magnification of 120,000 diameters we noted minute depressions on the surface of the erythroblast (Fig. 3 *a*). The membranes of these depressions form vacuoles which encompass 3 to 10 granules (Fig. 3 *b* and 3 *c*). The vacuoles are found on the cytoplasmic edge of the erythroblast and are from 300 to 800 mμ in diameter. An analogy between pinocytosis of Lewis and this mechanism is evident. The membrane encloses a drop of fluid which contains the granules. Nevertheless the granules eventually adhere to the internal membrane of the vacuole. We feel this process differs from phagocytosis (in which the pseudopods adhere directly to the surface of the substance to be engulfed) because in this case a liquid containing a solid is enclosed.

Hoffman *et al.* (4) have described a "new high density particle (H.D.P.)" in the stroma of erythrocytes obtained from patients with thalassemia, sicklemia, erythroblastosis, and as a normal component of the erythrocytes in some animals. They were unable to see H.D.P. in normal human erythrocytes, in the erythrocytes of iron-deficiency anemia, and in other animals. The technique utilized for the detection of the granules was hemolysis, with direct examination of the stroma. We feel that the H.D.P. described by the above authors is analogous to the iron-containing granules we have noted in sections of erythroblasts, erythrocytes, and siderocytes.

SUMMARY

Iron-containing granules (40 to 100 A in diameter), in groups or isolated, are present in normal erythroblasts, normoblasts, reticulocytes, and certain pathological erythrocytes. Similar granules are present in macrophages and have been noted in the stroma of erythrocytes following phagocytosis and hemolysis by macrophages. Incorporation of the granules from the macrophage into erythroblasts is presented as a process similar to pinocytosis. The presence of the iron-containing granules within the erythroid cells parallels hemoglobin synthesis.

REFERENCES

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4. Hoffman, J. F., Hillier, J., Wolman, W., and Parpart, A. K., *J. Cell. and Comp. Physiol.*, 1956, **47**, 245.
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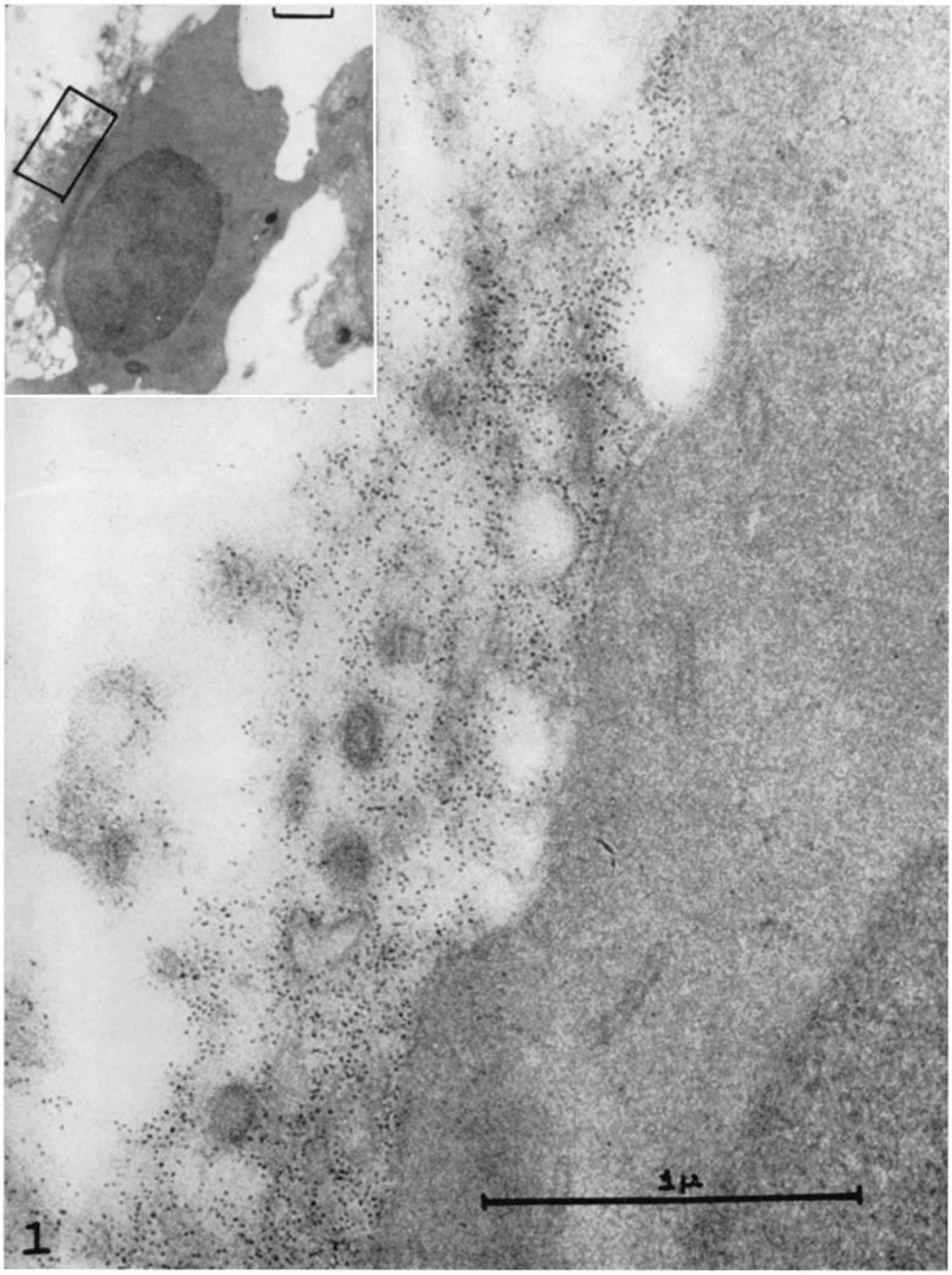
EXPLANATION OF PLATES

PLATE 161

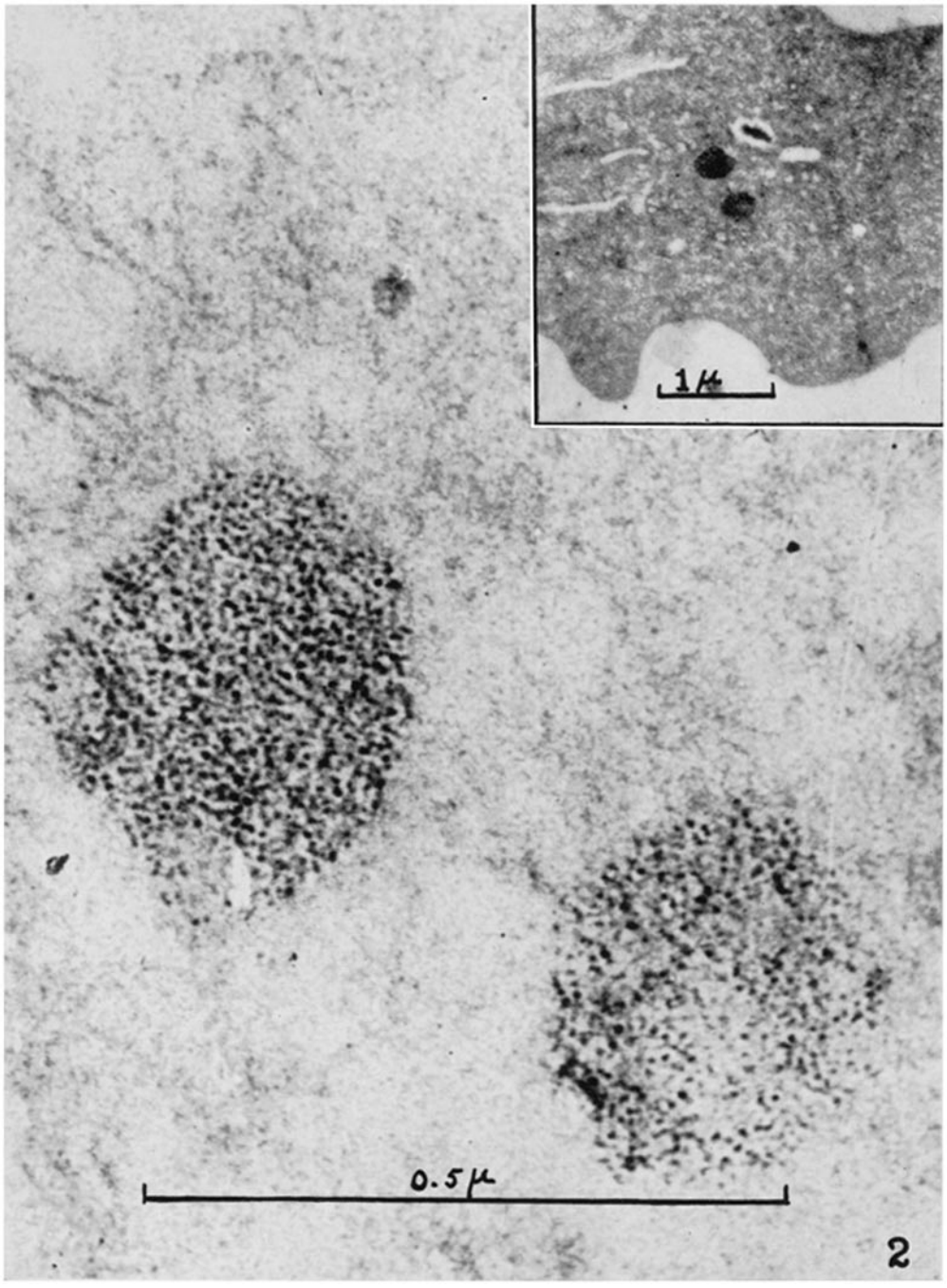
FIG. 1 and inset. Polychromatophilic normoblast with its border in contact with a reticular cell. Enlargement of the designated area showing numerous iron-containing granules in the reticular cell.



(Bessis and Breton-Gorius: Iron particles in erythroblasts)

PLATE 162

FIG. 2 and inset. Reticulocyte. Details of inset showing iron-containing granules accumulated in two large areas.



(Bessis and Breton-Gorius: Iron particles in erythroblasts)

PLATE 163

FIG. 3. Various aspects of the incorporation of iron-containing granules into the normoblast (the line indicates 0.1 μ).

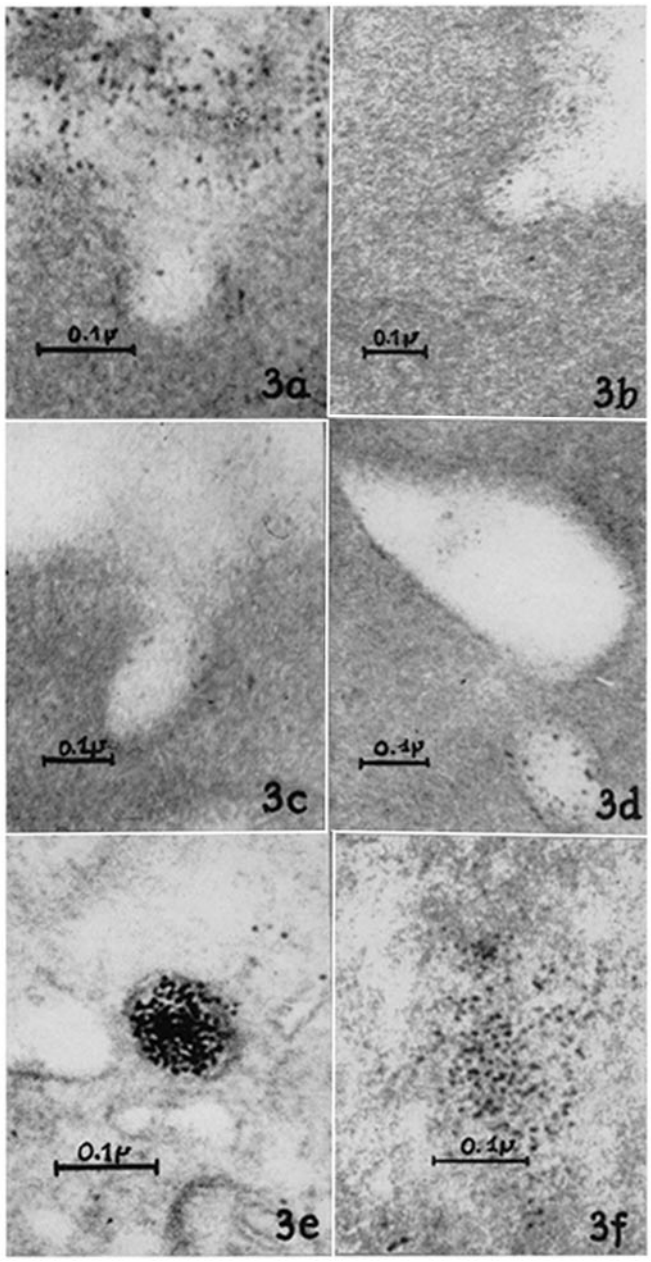
a. Cellular edge with a small depression.

b and *c*. A typical view of pinocytosis showing the enclosure of a few iron-containing granules.

d. A pinocytosis vacuole with iron-containing granules.

e. A mass of iron granules within the normoblast. These may be formed by fusion of numerous vacuoles.

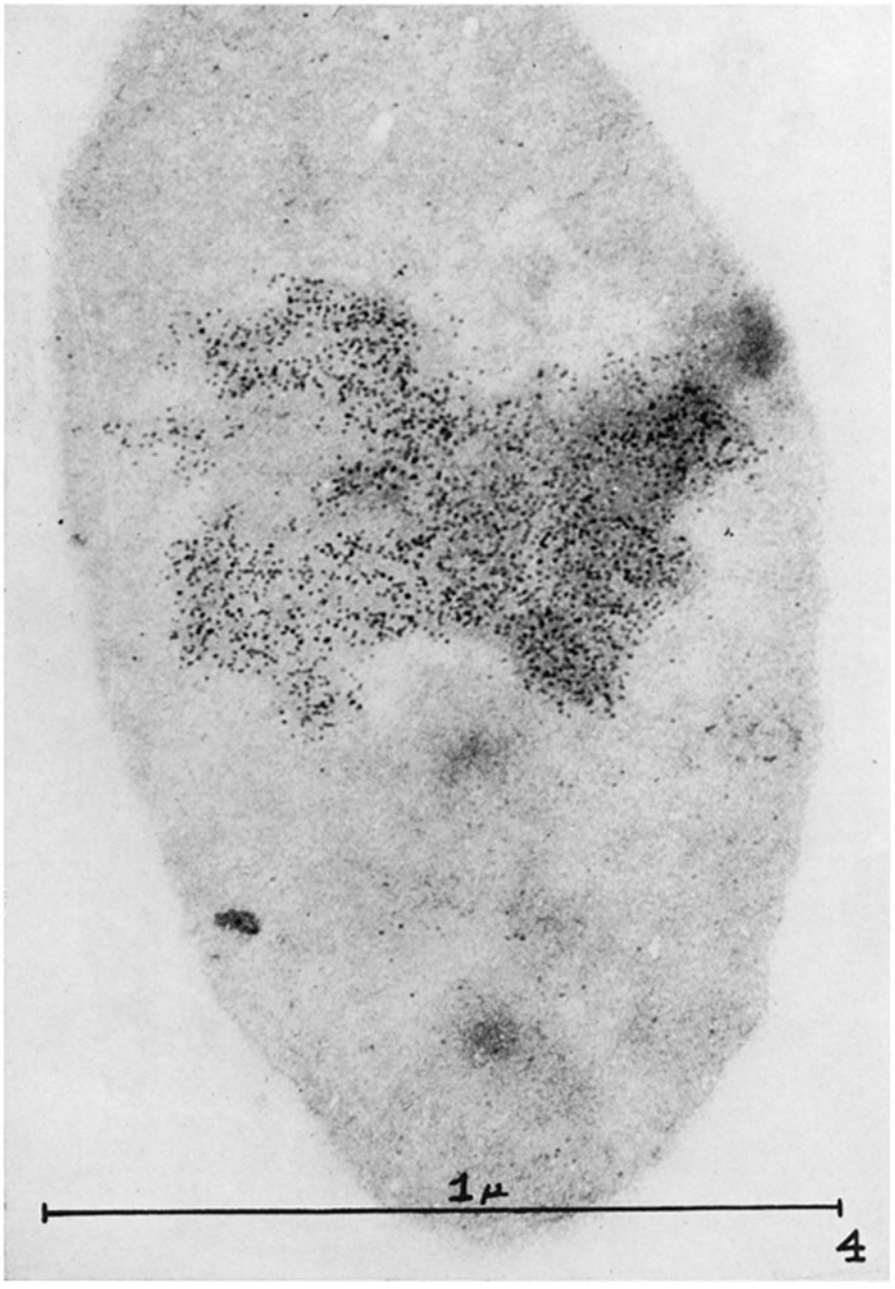
f. Dispersion of iron granules within the cytoplasm.



(Bessis and Breton-Gorius: Iron particles in erythroblasts)

PLATE 164

FIG. 4. Erythrocyte from thalassemia. Note the large mass of iron-containing granules enclosed within a discrete cytoplasmic zone plus the scattered isolated granules.



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